Practitioner's Docket No. ~~2260/103~~

CHAPTER II

Preliminary Classification:
Proposed Class:
Subclass:
NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P., § 601, 7th ed.

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US)

(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/IB99/00808	17 February 1999	18 February 1998
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED

PROCESS FOR THE OBTAINING OF HMG-CoA REDUCTASE INHIBITORS OF HIGH PURITY
TITLE OF INVENTION

PFLAUM et al.
APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231
ATTENTION: EO/US

CERTIFICATION UNDER 37 C.F.R. § 1.10*
(Express Mail label number is mandatory.)
(Express Mail certification is optional.)

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date 19 July 2000, in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL543499879US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Timothy M. Murphy
(type or print name of person mailing paper)

Signature of person mailing paper

WARNING: *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

****WARNING:*** *Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).*
*"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

NOTE: To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.

***WARNING:** Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing—See 37 C.F.R. § 1.8.*

NOTE: Documents and fees must be clearly identified as a submission to enter the national state under 35 U.S.C. § 371 otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).

I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. § 371:

a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).

b. ☒ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

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2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
☐*	TOTAL CLAIMS	23 −20=	3	× $18.00=	$ 54.00
	INDEPENDENT CLAIMS	2 −3=	0	× $78.00=	0
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	
BASIC FEE**	☐ U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an international preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: ☐ and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. § 1.492(a)(4)) ..... $96.00 ☐ and the above requirements are not met (37 C.F.R. § 1.492(a)(1)) ..... $670.00 ☒ U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: ☐ has been paid (37 C.F.R. § 1.492(a)(2)) ..... $690.00 ☐ has not been paid (37 C.F.R. § 1.492(a)(3)) ..... $970.00 ☒ where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. § 1.492(a)(5)) $840.00				840.00
	Total of above Calculations				= 894.00
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (note 37 C.F.R. § 1.9, 1.27, 1.28)				−
	Subtotal				
	Total National Fee				$ 894.00
	Fee for recording the enclosed assignment document $40.00 (37 C.F.R. § 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL	Total Fees enclosed				$ 894.00

*See attached Preliminary Amendment Reducing the Number of Claims.

i. ☒ A check in the amount of _____ to cover the above fees is enclosed.

ii. ☐ Please charge Account No. _____ in the amount of $ _____. A duplicate copy of this sheet is enclosed.

***WARNING:** *"To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).*

WARNING: *If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.*

3. ☒ A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

a. ☒ is transmitted herewith.

b. ☐ is not required, as the application was filed with the United States Receiving Office.

c. ☐ has been transmitted

i. ☐ by the International Bureau.
Date of mailing of the application (from form PCT/1B/308): _____.

ii. ☐ by applicant on _____.
Date

4. ☒ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

a. ☐ is transmitted herewith.

b. ☒ is not required as the application was filed in English.

c. ☐ was previously transmitted by applicant on _____.
Date

d. ☐ will follow.

5. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

a. ☒ are transmitted herewith.

b. ☐ have been transmitted

i. ☐ by the International Bureau.
Date of mailing of the amendment (from form PCT/1B/308): _____.

ii. ☐ by applicant on (date) _____.
Date

c. ☐ have not been transmitted as

i. ☐ applicant chose not to make amendments under PCT Article 19.
Date of mailing of Search Report (from form PCT/ISA/210.): _____.

ii. ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☒ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. § 371(c)(3)):

a. ☐ is transmitted herewith.

b. ☒ is not required as the amendments were made in the English language.

c. ☐ has not been transmitted for reasons indicated at point 5(c) above.

7. ☒ A copy of the international examination report (PCT/IPEA/409)

☒ is transmitted herewith.

☐ is not required as the application was filed with the United States Receiving Office.

8. ☐ Annex(es) to the international preliminary examination report

a. ☐ is/are transmitted herewith.

b. ☐ is/are not required as the application was filed with the United States Receiving Office.

9. ☐ A translation of the annexes to the international preliminary examination report

a. ☐ is transmitted herewith.

b. ☐ is not required as the annexes are in the English language.

10. ☒ An oath or declaration of the inventor (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115

a. ☐ was previously submitted by applicant on _____. Date

b. ☐ is submitted herewith, and such oath or declaration

i. ☐ is attached to the application.

ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. § 1.70.

c. ☒ will follow.

II. Other document(s) or information included:

11. ☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):

a. ☒ is transmitted herewith. (with IDS)

b. ☐ has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308): _____.

c. ☐ is not required, as the application was searched by the United States International Searching Authority.

d. ☐ will be transmitted promptly upon request.

e. ☐ has been submitted by applicant on _____. Date

12. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:

a. ☒ is transmitted herewith.

Also transmitted herewith is/are:

☒ Form PTO-1449 (PTO/SB/08A and 08B).

☒ Copies of citations listed.

b. ☐ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).

c. ☐ was previously submitted by applicant on _____. Date

13. ☐ An assignment document is transmitted herewith for recording.

A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

_____

_____

_____

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14. ☒ Additional documents:

a. ☐ Copy of request (PCT/RO/101)

b. ☒ International Publication No. WO 99/42601

i. ☒ Specification, claims and drawing

ii. ☐ Front page only

c. ☒ Preliminary amendment (37 C.F.R. § 1.121)

d. ☒ Other

1) Notification Concerning Submission or Transmittal of Priority Document

2) PCT Written Opinion

3) Response to Written Opinion

15. ☒ The above checked items are being transmitted

a. ☒ before 30 months from any claimed priority date.

b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on _____, namely:

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: *Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.*

NOTE: *"A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).*

NOTE: *"Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).*

☒ The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 19-4972.

☒ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: *Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.*

☒ 37 C.F.R. § 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

☒ 37 C.F.R. § 1.17 (application processing fees)

☒ 37 C.F.R. § 1.17(a)(1)–(5) (extension fees pursuant to § 1.136(a).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☐ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).

SIGNATURE OF PRACTITIONER

Reg. No.: 33,198

Timothy M. Murphy
(type or print name of practitioner)

Tel. No.: (617) 443-9292

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125 Summer Street, Boston, MA 02110

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Pflaum et al.	Att'y Docket:	2260/103
Int'l. Appln. No:	PCT/IB99/00808	Int'l. Filing Date:	February 18, 1998
Invention:	Process for the Obtaining of HMG-CoA Reductase Inhibitors of High Purity		

CERTIFICATE OF MAILING

I hereby certify that the following document is being transmitted via Express Mail EL543499879US to the Commissioner for Patents, Box PCT, Washington, D.C. 20231, Attn: EO/US on July 19, 2000.

Timothy M. Murphy

Commissioner for Patents
Box PCT
Washington, D.C. 20231
Attn: EO/US

PRELIMINARY AMENDMENT

Dear Sir:

The applicants submit this preliminary amendment in connection with entering the national phase for the above-referenced international patent application. Please enter the following amendment to the national stage application.

In the Description:

On page 1, line 22, after "period as", please delete "it".

On page 3, line 27, please replace "dissolvation" with --dissolution--.

On page 3, line 30, please replace "dissolvation" with --dissolution--.

On page 4, line 4, please replace "dissolvation" with --dissolution--.

On page 4, lines 11, please replace "dissolvation" with --dissolution--.

On page 4, line 16, please replace "dissolvating" with --dissolving--.

On page 5, line 31, please replace "pharma-ceutical" with --pharmaceutical--.

On page 6, line 1, please replace "raw" with --crude--.

On page 6, line 4, please replace "raw" with --crude--.

On page 6, line 10, please replace "raw" with --crude--.

On page 7, line 4, please replace "the man" with --one--.

In the Claims:

Please add the following new claims 24-47 and cancel claims 1-23 without prejudice:

24. (new) A process for the isolation and purification of HMG-CoA reductase inhibitors from mycelium biomass which comprises:

- clarifying a mycelium broth and concentrating the clarified broth to a lower volume,
- acidifying of the concentrate to a pH value in the range of 4.5 to 7.5, followed by extracting the HMG-CoA reductase inhibitor with ethyl acetate,
- optionally performing lactonization,
- crystallization of the HMG-CoA reductase inhibitor from a water-miscible or water-soluble organic solvent, and
- crystallization of the HMG-CoA reductase inhibitor from an organic solvent having limited miscibility or solubility with water.

25. (new) The process according to claim 24, further comprising, before clarifying the mycelium biomass broth:

- dissolving the HMG-CoA reductase inhibitor from a mycelium biomass at pH value between 9.5 and 13 into fermentation liquor, and
- adjusting the broth to a pH value between 7.5 and 8.5.

26. (new) The process according to claim 25, wherein the dissolving step is carried out at a

temperature in the range of 10 to 40°C for less than one hour.

27. (new) The process according to claim 24, wherein clarifying the mycelium broth is carried out by removing the mycelium from the broth by means of filtration.

28. (new) The process according to claim 24, wherein said clarified broth is concentrated by means of reverse osmosis.

29. (new) The process according to claim 24, wherein the concentrate is acidified to a pH value in the range of 5.5 to 7.5.

30. (new) The process according to claim 24, wherein the concentrate is acidified to a pH value in the range of 6.0 to 7.0.

31. (new) The process according to claim 24, wherein the HMG-CoA reductase inhibitor which is extracted from ethyl acetate and optionally lactonized is subjected to a purification step by adsorption chromatography.

32. (new) The process according to claim 31, wherein a mixture of acetonitrile and water is used as the mobile phase for adsorption chromatography.

33. (new) The process according to claim 24, wherein the order of the crystallization steps is reversed.

34. (new) The process according to claim 24, wherein the water-miscible or water-soluble organic solvent used in the crystallization step is acetone or a low alkyl alcohol.

35. (new) The process according to claim 24, wherein the crystallization step from a water-miscible or water-soluble organic solvent comprises dissolving the HMG-CoA reductase

inhibitor in acetone, and then adding water thereto.

36. (new) The process according to claim 24, wherein the crystallization step from an organic solvent having limited miscibility or solubility with water comprises dissolving the HMG-CoA reductase inhibitor in said organic solvent at a concentration of 10 to 35 g/l, and removing one-third to three-fourth of said organic solvent.

37. (new) The process according to claim 24, wherein the organic solvent having limited miscibility or solubility with water used in the crystallization step is ethyl acetate.

38. (new) The process according to claim 24, wherein HMG-CoA reductase inhibitors are obtained having a purity higher than 99.6%.

39. (new) The process according to claim 24, wherein the HMG-CoA reductase inhibitor is selected to be lovastatin.

40. (new) A process for the purification of HMG-CoA reductase inhibitors which comprises subjecting the HMG-CoA reductase inhibitor to combined crystallization steps, which comprises crystallization from an water-miscible or water-soluble and crystallization from an organic solvent having limited miscibility or solubility with water, as final polishing steps to obtain HMG-CoA reductase inhibitors having a purity higher than 99.6%.

41. (new) The process according to claim 40, wherein the obtained HMG-CoA reductase inhibitors have purity higher than 99.7 %.

42. (new) The process according to claim 40, wherein wherein acetone or a low alkyl alcohol is used as the water-miscible or water-soluble organic solvent.

43. (new) The process according to claim 40, wherein the crystallization from a water-miscible

or water-soluble organic solvent comprises dissolving the HMG-CoA reductase inhibitor in acetone, and then adding water thereto.

44. (new) The process according to claim 40, wherein said crystallization from an organic solvent having limited miscibility or solubility with water comprises dissolving the HMG-CoA reductase inhibitor in said organic solvent at a concentration of 10 to 35 g/l, and removing one-third to three-fourth of said organic solvent.

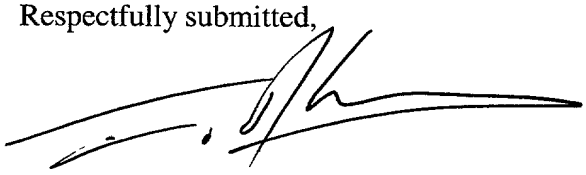
45. (new) The process according to claim 40, wherein ethyl acetate is used as the organic solvent having limited miscibility or solubility with water.

46. (new) Use of a process according to claim 40 for the isolation and/or purification of lovastatin.

REMARKS

The foregoing amendment is intended to remove the multiple claims and to place the claims in proper U.S. form. It is believed that the application is in condition for allowance.

Respectfully submitted,



Date: July 19, 2000

Timothy M. Murphy
Registration No. 33,198
Attorney for Applicants

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Tel: 617 443 9292
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02260/00001 122855.1

TITLE OF THE INVENTION

Process for the obtaining of HMG-CoA reductase inhibitors of high purity

Description

Background of the invention

Lovastatin, pravastatin, mevastatin, simvastatin, their derivatives and analogs are known as HMG-CoA reductase inhibitors and are used as antihypercholesterolemic agents. They are produced by fermentation using microorganisms of different species identified as species belonging to *Aspergillus, Monascus, Nocardia, Amycolatopsis, Mucor* or *Penicillium* genus.

Purity of the active ingredient is an important factor for the manufacturing of a safe and effective pharmaceutical. The highest possible purity of the product is especially important if the pharmaceutical product should be taken for a longer period as it is the case in the treatment or the preventing of a high plasma cholesterol. The accumulation of the impurities from the pharmaceuticals of lower purity can cause many side effects during the medical treatment.

The processes for the isolation and purification of the antihypercholesterolemic agents disclosed in the earlier patent applications comprise different combinations of extraction, chromatography, lactonization and crystallization methods. The purity of the final product obtained by these procedures is lower than 99.6%. Obtaining the product of higher purity by use of these methods is possible, but the yield of the desired product is then unacceptably low for using those methods in a large industrial scale.

The isolation process disclosed in patent application WO 92/16276 provides the solution for obtaining HMG-CoA reductase inhibitors of purity higher than 99.5%, but the use of highly sophisticated industrial high performance liquid chromatography (HPLC) equipment is required. According to the WO 92/16276 the crude HMG-CoA reductase inhibitor of approximately 85% or higher purity is dissolved in an organic solvent or in a solution of organic solvent and water. The mixture is then buffered to a pH between 2 and 9 and placed on an HPLC column. After the HMG-CoA reductase inhibitor peak of interest is collected, a portion of solvent is removed and then water is added or alternatively two-thirds of the solvent mixture are removed to crystallize the HMG-CoA reductase inhibitor. At the end the purity of the product achieved by this process is really at least 99.5% with yield of approximately 90%.

Summary of the invention

The present invention relates to a new industrial process for isolation and purification of HMG-CoA reductase inhibitors of purity higher than 99.6% and preferably higher than 99.7 % from a fermentation broth. To achieve this goal an extensive study of the chemical compounds produced during the fermentation using the different species of microorganisms belonging to *Aspergillus, Monascus, Nocardia, Mucor, Amycolatopsis* or *Penicillium* genus, their chemical properties and their behavior in the different solvents at different pH was done. Thus, the aforementioned object was solved by the process of the present invention which comprises the following steps:

- clarifying a mycelium broth and concentrating the clarified broth to a lower volume,
- acidifying of the concentrate to a pH value in the range of 4.5 to 7.5, followed by extracting the HMG-CoA reductase inhibitor with ethyl acetate,

- optionally performing lactonization,
- performing crystallization of the HMG-CoA reductase inhibitor from a water-miscible or water-soluble organic solvent, and
- performing crystallization of the HMG-CoA reductase inhibitor from an organic solvent having limited miscibility or solubility with water.

Detailed description of the invention

Referring to the drawings,
Fig. 1 shows the dependency from the pH of the distribution coefficient of a HMG-CoA reductase inhibitor (lovastatin) and of impurities, respectively, in the ethyl acetate extraction step, and
Figs. 2, 3 and 4 show HPLC diagrams of samples of HMG-CoA reductase inhibitor after ethyl acetate extraction as a crude composition, after crystallization from a water-miscible or water-soluble organic solvent, and after further crystallization from an organic solvent having limited miscibility or solubility with water, respectively.

Since HMG-CoA reductase inhibitors are typically both intra- and extracellular products, it is not mandatory but preferable to dissolve them effectively from the mycelium into fermentation liquor. The method for dissolvation disclosed in patent application WO 97/20834 comprises treatment of fermentation broth with alkaline base to pH 11.5 and stirring for three hours. The WO 97/06128 teaches that dissolvation may be done with alkalifying of the fermentation broth to pH between 10 and 13. Also a temperature between 60 and 95 °C is applied. HMG-CoA reductase inhibitors may be very efficiently dissolved from the mycelium at a pH higher than 9, but too long exposure to so rigorous condition causes the degradation of ester bond between hydroxyl group on naphthalene skeleton and carboxylic acid. Equilibrium between

HMG-CoA reductase inhibitors and deacylated HMG-CoA reductase inhibitors shifts at more rigorous conditions to deacylated products. We have unexpectedly found out that the efficiency of dissolvation carried out in a temperature range of 10 to 40°C, preferably in the range of 18 to 25°C, such as room temperature, for less than one hour, preferably for less than half an hour, for example for about 10 minutes, at a pH between 9.5 and 13, most preferably between 9.5 and 11.5, is equal to the efficiency achieved by less economic and more time consuming methods carried out at higher temperatures described in earlier patent applications. The dissolvation may be carried out also at a pH lower than 9.5 and especially lower than 6, but the use of a huge amount of organic solvents is necessary in this case.

If this preferred embodiment of dissolvating the HMG-CoA reductase inhibitor has been carried out, the fermentation broth is subsequently treated with an acidifying agent, suitably with mineral acid, to adjust the pH value between 7.5 and 8.5. Suitable mineral acids are phosphoric, sulfuric and hydrochloric acid. HMG-CoA reductase inhibitors are stable in this range of pH and the fermentation broth can be also stored for a while after this step, if that is necessary or desired.

The mycelium is removed from the fermentation broth by means of appropriate separation steps, such as filtration and/or centrifugation. Filtration is preferred, and as a filtration technique beside classic filtration also micro-, ultra- and diafiltration may suitably be used. The clarified broth is then concentrated to a lower volume, most preferably five to ten times, by means of reverse osmosis or some other methods for lowering volume.

The acidification and ethyl acetate extraction step described in the following is a significant point of the purification process.

The said concentrate is acidified by an acidifying agent, suitably with mineral acid, to a pH value between 4.5 and 7.5. Mineral acids already mentioned above as examples can be used. Then, HMG-CoA reductase inhibitor is extracted from the said pH-adjusted concentrate with ethyl acetate. Extraction is suitably done by using a counter-current extraction column. The ratio between distribution coefficients of HMG-CoA reductase inhibitors and ethyl acetate soluble impurities is the highest at a pH value from 5.5 to 7.5 and especially at a pH value from 6.0 to 7.0, and a part of polar impurities is already removed at this step. The extraction carried out at pH value lower than 5.0, especially lower than 4, is more efficient, because of higher distribution coefficient of HMG-CoA reductase inhibitors, but it results in high level of polar impurities. The distribution coefficients of ethyl acetate soluble impurities are also high at that pH value, as is shown in Fig. 1. The extraction into ethyl acetate carried out at a pH value between 4.5 and 7.5, especially above 5.0 and in particular above 5.5, results in lower level of polar impurities because of their low distribution coefficients. Worse distribution of HMG-CoA reductase inhibitors from the concentrate into ethyl acetate at that pH value can be compensated with a longer counter-current extraction column.

If desired, the resulted ethyl acetate extract is then concentrated and HMG-CoA reductase inhibitor is lactonized optionally at this stage of the process. At pH between 5.5 and 7.5, the major part of the HMG-CoA reductase inhibitor is in free acid form. Therefore, the concentration and lactonization may be omitted if HMG-CoA reductase inhibitor is not used in the pharma-ceutical as a lactone. The lactonization is suitably done by contacting the HMG-CoA reductase inhibitor with catalytic amount of mineral or organic acid, most preferably trifluoracetic acid (TFA). The HMG-CoA reductase inhibitor which is optionally lactonized may then be directly crystallized from ethyl acetate, as will be described below. Alternatively, the ethyl acetate is removed, suitably by

vaporization, and a raw HMG-CoA reductase inhibitor product, which is optionally lactonized, is obtained.

The thus obtained raw HMG-CoA reductase inhibitor may then optionally be subjected to adsorption chromatography, preferably to reversed phase chromatography. As the mobile phase for adsorption chromatography, acetonitrile or lower alcohols such as methanol, ethanol or propanol, or a mixture of these solvents with water, can suitably be used. Preferably, the raw HMG-CoA reductase inhibitor is dissolved in pure acetonitrile or mixture acetonitrile/water with at least 30% volume/volume (v/v) of acetonitrile, and the resulting solution is placed on an adsorption chromatography column. The column packing include, but are not limited to stationary phases based on octylsilane, dimethylsilane, octadecylsilane, cyano-silane, polystyrenedivinylbenzene copolymer or acrylic polymer. Other typical stationary phase materials may also be used, for example silica, alumina, or the like. The adsorbed compounds are eluted with an appropriate mobile phase, such as acetonitrile/water gradient. The HMG-CoA reductase inhibitor peak of interest is collected and the mobile phase solvent is removed to crystallize the HMG-CoA reductase inhibitor. The purity of crystallized crude HMG-CoA reductase inhibitors is between 80% and 92% and depends on impurity profile in the fermentation broth. The optional adsorption chromatography may also be replaced by normal chromatography, flash chromatography, industrial HPLC, or by methods of extraction or crystallization.

The combined crystallization treatment which is peculiar according to the present invention will be described in more detail in the following.
More specifically, it comprises crystallization of the HMG-CoA reductase inhibitor from an organic solvent being water-miscible or water-soluble, and crystallization of the HMG-CoA reductase inhibitor from an organic solvent having a limited miscibility or solubility with water. The order of both

crystallizations may also be inverse. The property of the organic solvent of being either water-miscible or water-soluble, or having a limited miscibility or solubility with water is per se known to the man skilled in the art and is, for example, described in "Ullmann's Encyclopedia of Industrial Chemistry", Vol. A24, 5th edition (1993), pp. 437-505, incorporated herein by reference. In the meaning of the present invention, the term "water-miscible or water-soluble" shall refer to organic solvents which show essentially unlimited, preferably 100 % miscibility or solubility with water, and the term "limited miscibility or solubility with water" shall also include water-immiscible or water-insoluble organic solvents. Furthermore, the concept of crystallization of the present invention in particular also includes precipitation.

Examples for essentially water-miscible or water-soluble organic solvents include: low alkyl alcohols such as methanol, ethanol, propanol and isopropyl alcohol, low alkyl ketones such as acetone and methyl ethyl ketone, low alkyl glycol ethers such as methyl glycol, ethyl glycol, propyl glycol and ethyl diglycol, and dipolar aprotic solvents such as N,N-dimethyl-formamide (DMF), N,N-dimethylacetamide (DMA) and dimethyl sulfoxide (DMSO), including mixtures of these solvents. As particularly preferred examples for the water-miscible organic solvent, acetone and low alkyl alcohols are mentioned. Examples for an organic solvent having limited miscibility or solubility with water include: higher alkyl alcohols such as butanol, iso-butanol amyl alcohol, hexanol, 2-ethylhexanol, benzyl alcohol and cyclohexanol, higher alkyl ketones such as methylbutyl ketone, methyl isobutyl ketone and cyclohexanone, esters such as methyl acetate, ethyl acetate, n-propyl (and isopropyl) acetate, n-butyl (and iso-butyl or sec-butyl) acetate and amyl acetate, ethers such as diethyl ether and diisopropyl ether, chlorinated hydrocarbons such as methylene chloride and chloro-form, acetonitrile and the like, including mixtures of these solvents. Particularly preferred as a solvent having limited miscibility or solubility with water is ethyl acetate.

We have unexpectedly found out that the crystallization of HMG-CoA reductase inhibitors from water-miscible organic solvent like acetone or low alkyl alcohol followed by further recrystallizations with the same solvent can remove only a minor part of nonpolar and a major part of polar impurities, and the crystallization from organic solvent having a limited miscibility with water like ethyl acetate followed by further recrystallizations from the same solvent removed only major nonpolar impurities. The last fact is clearly evident from HPLC diagrams of crude HMG-CoA reductase inhibitor (Fig. 2), HMG-CoA reductase inhibitor after the crystallization from acetone (Fig. 3) and HMG-CoA reductase inhibitor obtained by the crystallization from acetone and further recrystallized from ethyl acetate (Fig. 4). According to this unexpected recognition, the last step of the present invention comprising combined crystallization from water-miscible or water-soluble organic solvent and from an organic solvent having limited miscibility or solubility with water cannot be omitted in the process for achieving HMG-CoA reductase inhibitors of high purity.

The combined crystallization treatment according to the present invention may be effected as follows. First, the crystals of crude HMG-CoA reductase inhibitor are dissolved in the afore-mentioned substantially (preferably 100 %) water-miscible or water-soluble organic solvent, in particular acetone or lower alcohol, and then water is added to let HMG-CoA reductase inhibitor crystallize or precipitate. Alternatively, the crude HMG-CoA reductase inhibitor being dissolved in the substantially water- miscible or water-soluble organic solvent is added to water for being crystallized or precipitated. These procedures may be repeated again with the same or another water-miscible or water-soluble organic solvent, if necessary, for example from one to four times depending on the purity of the starting crude material.

The crystals obtained thereby are then dissolved in the afore-mentioned solvent having limited miscibility or solubility with water, like ethyl acetate, to an appropriate concentration which preferably lies in the range of 10 to 35 g/l, most preferably in the range of 15 to 25 g/l. After the removal of one-third to three-fourth of solvent, the HMG-CoA reductase inhibitor crystallizes. Crystallization from the same or another organic solvent having limited miscibility or solubility with water may be repeated, if necessary, for example for one to three times depending on the purity of the product obtained by crystallization from water-miscible or water-soluble organic solvent. The crystallized HMG-CoA reductase inhibitor is then filtered and dried to yield a product of purity of at least 99.6 %.

As already mentioned, the order of crystallizations may be inverse, i.e. first performing crystallization from the organic solvent having limited miscibility or solubility with water, and then performing crystallization from the water-miscible or water-soluble organic solvent. In a preferred embodiment of the present invention, first performing crystallization from ethyl acetate as the organic solvent having limited miscibility or solubility with water may suitably be effected directly after the ethyl acetate extraction step or, optionally, after the lactonization step described above.

With the process according to the present invention, products having a purity of at least 99.6% and even at least 99.7 % are achievable.

In a further alternative embodiment, the different kinds of crystallizations may be performed repeatedly in an alternating manner.

In another aspect of the present invention, the previously described process of combined crystallization steps from water-miscible or water-soluble organic solvent and from organic

solvent having limited miscibility or solubility with water are employed as a final polishing step of any process for isolation and/or purification of HMG-CoA reductase inhibitors. Accordingly, such a final polishing step can also be applied to raw materials of HMG-CoA reductase inhibitor which have been conventionally obtained. The thus achievable purity of HMG-CoA reductase inhibitor is at least 99.6 % and even at least 99.7 %.

The process according to the present invention is well suited especially when lovastatin is selected as the HMG-CoA reductase inhibitor. Accordingly in another aspect of the present invention, the process described above is used for the isolation and/or purification of lovastatin.

The essentially pure HMG-CoA reductase inhibitors obtained by the process according to the present invention, such as lovastatin, mevastatin, pravastatin and simvastatin as well as their derivatives and analogues, can be beneficially used for the preparation of a pharmaceutical for the prevention and/or treatment of diseases. The obtained inhibitors and pharmaceuticals are particularly useful as medicaments or preventives for reducing the risk of stroke, transient ischemic attack, atherosclerosis and myocardial infarction.

The following examples illustrate the process of the instant invention and are not to be considered as limiting the invention set forth in the claims appended hereto.

EXAMPLES

Example 1

Fermentation broth (160 l) with concentration of lovastatin 1 g/l obtained by fermentation with *Aspergillus terreus* ATCC 20542 was placed into the vessel (400 l) and

adjusted to pH 10 with 1 M aqueous sodium hydroxide solution. After 10 minutes of intensive stirring at room temperature the broth was adjusted to pH 9 with 1 M sulfuric acid solution and the biomass was filtered off. The filtrate was then acidified with 1 M sulfuric acid solution to pH 6.5. 160 l of ethyl acetate was added to filtrate and the obtained mixture was stirred for 20 min. The aqueous and ethyl acetate phases were separated by extraction centrifuge. The ethyl acetate extract was concentrated in rotary evaporator to volume of 14 l. The concentration of the lovastatin in the free acid form in the ethyl acetate concentrate amounted to 10 g/l.

The ethyl acetate concentrate (14 l) was then placed into reactor (40 l) and lactonized. The lactonization was initiated by catalytic amount of TFA (0.5 ml of TFA/ 1 l of concentrate). The lactonization procedure lasted for two hours at 40 °C. The concentrate was washed after the lactonization two times with 14 l of 5 % ammonium hydrogen carbonate aqueous solution. The aqueous phase was discharged, the organic phase was further concentrated to dry in rotary evaporator. The resulted oily product (1.5 l) contained 133 g of lovastatin.

The obtained oily product (161 ml) was dissolved in 80 ml of acetonitrile and loaded on a chromatography column (80 cm, 3.6 cm) filled with XAD-16 (XAD-16 is the commercial name of company Rohm & Hass, 20-50 mesh). The column was eluted first with 40:60 acetonitrile/water (pH 3, adjusted by hydrochloric acid) at a rate of 75 ml/min. Elution was monitored by UV detector (236 nm) and after first drop of absorption the elution of the column with 55:45 acetonitrile/water (pH 3, adjusted by hydrochloric acid) was started. The main fraction was collected and after the fall of the absorption the column was washed with 80:20 acetonitrile/water (pH 3, adjusted by hydrochloric). The acetonitrile was removed from the main fraction by rotary evaporator (50 °C, 150 mbar) and the resulted crystals were filtered off. Mass of crystals was 24.5

g and the content of lovastatin was 50 % weight/weight (w/w). HPLC purity was 92.5 %.

Resulted crystals (24 g) were dissolved in 350 ml acetone and 700 ml water was added under continuos stirring. The mixture was placed on 4 °C for 30 minutes. Obtained crystals were filtered off and dried in vacuo at room temperature. Mass of crystals was 12.7 g with the content 90 % w/w of lovastatin. HPLC purity was 98.8 %.
The crystallization from acetone was repeated under the same condition and 11.3 g of crystals with 97 % w/w of lovastatin were obtained. HPLC purity was 99.4 %.

The crystals (11.3 g) obtained after the second crystallization from acetone were dissolved in 700 ml of ethyl acetate and the ethyl acetate was evaporated in vacuo to the concentration of lovastatin 70 g/l. The concentrate was placed on 8 °C for one hour. Resulted crystals of lovastatin were filtered off and then dried in vacuo. Mass of crystals was 9.4 g with 99.6 % w/w content of lovastatin. HPLC purity was 99.7 %.

Example 2

Lovastatin crystals (3 g), isolated after the XAD-adsorption chromatography as described in Example 1, were dissolved in 170 ml ethyl acetate. The ethyl acetate was evaporated in vacuo (200 mbar) at 50 °C to 35 ml. The concentrate was placed on 10 °C for one hour. Resulted crystals of lovastatin were filtered off and then dried in vacuo. Mass of crystals was 2.1 g with 96 % w/w content of lovastatin. HPLC purity was 99.0 %.

The obtained crystals (2.1 g) were dissolved in 50 ml acetone and 85 ml water was added. The mixture was placed then on 10 °C for 30 minutes and the crystals were filtered off and

dried in vacuo at 40 °C. Mass of resulted crystals was 1.9 g with the 99 % w/w of lovastatin. HPLC purity was 99.8 %.

Example 3

A fermentation broth (30 l in a 50 l fermentor) containing pravastatin (690 g per kg of fermentation broth; HPLC purity of pravastatin was 48.7 %) was filtered and the resulting mycelium was washed with water. Filtrate (51 l) was acidified to pH 5.0 with 10 % aqueous solution of phosphoric acid. Active substance (pravastatin) was then extracted in an extraction column from the filtrate into 70 l of ethyl acetate. Water phase (50 l) with less than 2 g of pravastatin and with major part of impurities was discharged. The ethyl acetate phase was evaporated to 800 ml and used further in the process of isolation. The HPLC purity of pravastatin in the ethyl acetate extract was 70.3 %.
For further isolation, the oily product was subjected to adsorption chromatography and combined crystallization steps in accordance with Example 1.

Example 4

A crude simvastatin (2.3 g) in lactone form was dissolved in acetone (7 ml) and 15 ml of water was added. The result was oily product that crystallized next in 10 minutes. The obtained crystals were then filtered, washed with water and dried at 40 °C for 60 min. The resulted crystals (2.2 g) with HPLC purity of 99.51 % were then dissolved in ethyl acetate (8 ml). The resulted solution was concentrated to 4 ml, and simvastatin was left to crystallize for 60 min at 8 °C. The product was filtered and washed with water. The crystals were then dried at 40 °C for 60 min. The purity of the resulted simvastatin (1.7 g) was 99.73 %.

Example 5

A crude mevastatin (2.0 g) in lactone form with HPLC purity 98.5 % was dissolved in acetone (7 ml) and 20 ml of water was added. The result was oily product that crystallized next in 10 min. The obtained crystals were then filtered, washed with water and dried at 40 °C for 60 min. The resulted crystals (1.8 g) with HPLC purity 99.33 % were then dissolved in ethyl acetate (8 ml). The resulted solution was concentrated to 4 ml and mevastatin was left to crystallize for 60 min at 8 °C. The product was filtered and washed with water. The crystals were then dried at 40 °C for 60 min. The purity of the resulted mevastatin (1.3 g) was 99.72 %.

Claims

1. A process for the isolation and purification of HMG-CoA reductase inhibitors from a mycelium biomass which comprises:
- clarifying a mycelium broth and concentrating the clarified broth to a lower volume,
- acidifying of the concentrate to a pH value in the range of 4.5 to 7.5, followed by extracting the HMG-CoA reductase inhibitor with ethyl acetate,
- optionally performing lactonization,
- crystallization of the HMG-CoA reductase inhibitor from a water-miscible or water-soluble organic solvent, and
- crystallization of the HMG-CoA reductase inhibitor from an organic solvent having limited miscibility or solubility with water.

2. The process according to claim 1, further comprising, before clarifying the mycelium biomass broth, the steps of dissolvating the HMG-CoA reductase inhibitor from a mycelium biomass at pH value between 9.5 and 13 into fermentation liquor, and adjusting the broth to a pH value between 7.5 and 8.5.

3. The process according to claim 2, wherein the dissolvation step is carried out at a temperature in the range of 10 to 40°C for less than one hour.

4. The process according to any one of the preceding claims, wherein clarifying the mycelium broth is carried out by removing the mycelium from the broth by means of filtration.

5. The process according to any one of the preceding claims, wherein said clarified broth is concentrated by means of reverse osmosis.

6. The process according to any one of the preceding claims, wherein the concentrate is acidified to a pH value in the range of 5.5 to 7.5.

7. The process according to claim 6, wherein the concentrate is acidified to a pH value in the range of 6.0 to 7.0.

8. The process according to any one of the preceding claims, wherein the HMG-CoA reductase inhibitor which is extracted from ethyl acetate and optionally lactonized is subjected to a purification step by adsorption chromatography.

9. The process according to claim 8, wherein a mixture of acetonitrile and water is used as the mobile phase for adsorption chromatography.

10. The process according to any one of the preceding claims, wherein the order of the crystallization steps is reversed.

11. The process according to any one of the preceding claims, wherein the water-miscible or water-soluble organic solvent used in the crystallization step is acetone or a low alkyl alcohol.

12. The process according to claim 11, wherein the crystallization step comprises dissolving the HMG-CoA reductase inhibitor in acetone, and then adding water thereto.

13. The process according to any one of the preceding claims, wherein the crystallization step from an organic solvent having limited miscibility or solubility with water comprises dissolving the HMG-CoA reductase inhibitor in said organic solvent at a concentration of 10 to 35 g/l, and removing one-third to three-fourth of said organic solvent.

14. The process according to any one of the preceding claims, wherein the organic solvent having limited miscibility or

solubility with water used in the crystallization step is ethyl acetate.

15. The process according to any one of the preceding claims, wherein HMG-CoA reductase inhibitors are obtained having a purity higher than 99.6%.

16. The process according to any one of the preceding claims, wherein the HMG-CoA reductase inhibitor is selected to be lovastatin.

17. A process for the purification of HMG-CoA reductase inhibitors which comprises subjecting the HMG-CoA reductase inhibitor to combined crystallization steps, which comprises crystallization from an water-miscible or water-soluble and crystallization from an organic solvent having limited miscibility or solubility with water, as final polishing steps to obtain HMG-CoA reductase inhibitors having a purity higher than 99.6%.

18. The process according to claim 17, wherein the obtained HMG-CoA reductase inhibitors have purity higher than 99.7 %.

19. The process according to claim 1 or 18, wherein wherein acetone or a low alkyl alcohol is used as the water-miscible or water-soluble organic solvent.

20. The process according to claim 19, wherein said crystallization comprises dissolving the HMG-CoA reductase inhibitor in acetone, and then adding water thereto.

21. The process according to any one of claims 17 to 20, wherein said crystallization from said organic solvent having limited miscibility or solubility with water comprises dissolving the HMG-CoA reductase inhibitor in said organic solvent at a concentration of 10 to 35 g/l, and removing one-third to three-fourth of said organic solvent.

22. The process according to any one of claims 17 to 21, wherein ethyl acetate is used as the organic solvent having limited miscibility or solubility with water.

23. Use of a process according to claim 1 or a process according to claim 17 for the isolation and/or purification of lovastatin.

Abstract

A process for the isolation and purification of HMG-CoA reductase inhibitors from a mycelium biomass is described, which process comprises: clarifying a mycelium broth and concentrating the clarified broth to a lower volume, acidifying of the concentrate to a pH value in the range of 4.5 to 7.5, followed by extracting the HMG-CoA reductase inhibitor with ethyl acetate, crystallization of the HMG-CoA reductase inhibitor from a water-miscible or water-soluble organic solvent, and crystallization of the HMG-CoA reductase inhibitor from an organic solvent having limited miscibility or solubility with water. The crystallization steps may also be reverse. The concept of a combination of the specified crystallization steps can also be used for the purification of a crude HMG-CoA reductase inhibitor.

Docket No.
2260/103

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROCESS FOR THE OBTAINING OF HMG-CoA REDUCTASE INHIBITORS OF HIGH PURITY

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **17 February 1999** as United States Application No. or PCT International Application Number **PCT/IB99/00808**

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
PCT/IB99/00808	**Slovenia**	**17 February 1999**	☐
(Number)	(Country)	(Day/Month/Year Filed)	
P-9800046	**Slovenia**	**18 February 1998**	☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

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(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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